Analysis of drugs of abuse metabolites using passive sampling and ultrahigh-liquid chromatography coupled to mass spectrometry

Carolina A. Jiménez-Rojas [a*]; Carlos Emilio Gutiérrez Ulloa [b]; Diego Rico [c]; Jaime A. Lara-Borrero [d]

[a;d] Civil Engineering, Pontificia Universidad Javeriana, Bogotá, Colombia; [b] Research leader, Corporación Autónoma Regional de Cundinamarca, Mosquera, Colombia; [c] Chemist, Universidad Nacional de Colombia, Bogotá, Colombia

Correspondence: Calle 146 #12-72, 905, Bogotá, Colombia; Phone: +573162879443; Email: carolinaa_jimenez@javeriana.edu.co

[a] Chemical Engineer, Universidad EAN. Master's degree candidate in hydrosystems, Pontificia Universidad Javeriana. Bogotá, Colombia; [b] Chemist, Universidad Nacional de Colombia. Postdoc: Texas Christian University (Dallas-USA) and Institute of Chemical Biology and Fundamental Medicine. PhD in Chemistry, Universidad de Alcalá. Master in molecular inorganic chemistry, Universidad Autónoma de Madrid. Research group leader - Dirección de Laboratorio e Innovación Ambiental, Corporación Autónoma Regional de Cundinamarca– CAR, Mosquera, Colombia; [c] Chemist, Universidad Nacional de Colombia. Master of Science - Chemistry, Universidad Nacional de Colombia. Analista de Cromatografía – Dirección de Laboratorio e Innovación Ambiental, Corporación Autónoma Regional – CAR, Mosquera, Colombia; [d] Civil Engineer, Pontificia Universidad Javeriana. Ph.D. in Roads, channels and ports engineering, Universidad Politécnica de Madrid. Master in Engineering and Management of the environment, Universitat Politècnica de Catalunya. Full proffesor, Pontificia Universidad Javeriana. Bogotá Colombia

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The present study proposes the monitoring of metabolites of drugs of abuse through the use of passive samplers in water systems. Initially, four positive ion metabolites of interest were determined according to national surveys, then composite sampling and passive sampling were implemented using the continuous flow passive sampler equipment with two types of sorbents inside, Empore Disk and Gerstel Twister. Two study sites were determined at the beginning and at the end of the middle Bogotá River basin after 4 days the sorbents were removed so that they could be desorbed and analyzed using UHPLC-MS methodology in laboratory. For composite samplings, results were below the FCCP of the chromatographical method and for passive sampling, peaks of benzoylecgonine (21427.3 pg mL^{-1}), methamphetamine (67101.5 pg mL^{-1}), ecstasy (225844.8 pg mL^{-1}) and methadone (15908.4 pg mL^{-1}) were found, allowing the postulation of passive sampling as an alternative to composite sampling in the monitoring of metabolites.

Keywords: Emerging pollutants, drugs of abuse, metabolites, passive sampling, CFIS, river, liquid chromatography

Introduction

Water resources are affected by substances that are difficult to treat, such as emerging pollutants (EPs). Due to their characteristics (produced by anthropogenic activities) and trace levels of concentration, EPs are dangerous for living beings and their surrounding ecosystems. [1]. EPs include different types of compounds such as surfactants, antibiotics, and other pharmaceuticals, polyhydroxyalkanoates (PHAs) and polychlorinated biphenyls (PCBs), hormones and other endocrine disruptors (EDCs), disinfection products, pesticides, drugs of abuse and their metabolites, natural algal toxins, personal care products (PCPs); among others. [2, 3].

Of the compounds that are considered EP, psychoactive substances have affected society by far, although they have been used for the treatment of pain for centuries [4],

their misuse has become a public health and environmental problem and they have become illicit drugs.

Worldwide, investigations were carried out in different social scenarios where EP – Illicit drugs were detected [5–7]. An example of this has been quantifying and estimating illicit drug use in concerts, music festivals, hospitals, prisons, holiday seasons, cities, and even supranational circumstances [7–13]. These studies have analyzed wastewater samples through spot or discrete sampling or composite sampling and have captured only point data on pollutant concentrations at the time of sampling, as is the case of the most recent study in Colombia conducted by Bijlsma et al.[14].

The principal difficulty of measuring EPs in general, in particular drugs of abuse, is related to sampling techniques. As the concentration of pollutants appears in trace levels, they can go unnoticed in discrete or even composite sampling, and because they take water samples at a point in time, it is difficult to capture peaks of contaminants. [15].

After sampling, EPs, need a quantification technique using different kinds of instrumental analysis equipment and methods, the decision must be made depending on the substance volatility. As for drugs of abuse and their metabolites, the methods of analysis are based on liquid chromatography coupled to mass spectrometry, among other associated lines (UHPLC, tandem spectrometry, etc.), as was the case of the first study conducted by Castigioni et al[16] whom in 2006 created an analysis methodology that has been a reference in Europe for the analysis of metabolites of drugs of abuse using LC-MS/MS in water samples.

However, the results showed that even if the chromatographic technique is adequate, the sampling methodology can be a source of interference in the

concentrations of these pollutants, as peaks of pollutants can be overlooked, and human errors can happen, among others.

One solution is to increase the sampling frequency or install automatic systems to take numerous water samples over a given period of time [17]. Nevertheless, this is costly and impractical as it requires a secure site and pre-treatment of the water (which is rarely done in monitoring campaigns) to avoid damage to the device [18].

Despite the above, passive samplers exist as an alternative to traditional and automatic systems, they can operate analogously to bioaccumulating organisms, and use sorbents that continuously retain the compounds of interest for days or even weeks. They can be useful for monitoring various types of EPs, such as metals, pesticides, PHAs and PCBs. Their advantages include the possibility of changing the sorbent, decreasing the impact on the solution in which they are found, obtaining time-weighted average (TWA) concentration values, their cost-effectiveness as they require less sampling and laboratory analysis~~,~~ and decreased sample degradation during transport and storage compared to other sampling techniques [19, 20, 15].

First models of passive samplers performed satisfactorily in sampling and sample collection but had limitations with turbulence, single capture of the soluble fraction, and specific to a single type of compound [21].

Subsequently, a new brand of passive samplers was developed: The continuous flow integrated samplers (CFIS), this sampler is a cost-effective alternative for monitoring variations in EP concentration [22]; is easy to operate in the field, it can preserve the shelf life of the collected sample, it cannot be affected by turbulence, it is capable of analyzing both the soluble and particulate fractions of a wide range of polar, non-polar and volatile pollutants, do not require sorbent pre-calibration, it is independent of the exposure conditions of the aquatic environment and it has

demonstrated lower detection limits than spot or composite sampling, providing more accurate and reliable contamination data [21, 23, 22, 24].

Notwithstanding the previous advantages, and to the best our knowledge, CFIS devices have not been used for monitoring emerging contaminants related to drugs of abuse.

Since frequent and timely information is needed to address the problem of emerging contaminants, the present article aims to conduct a study on the potential use of passive samplers in the detection of metabolites of drugs of abuse.

Materials and methods

Study zone

According to the regional environmental authority - Regional Autonomous Corporation of Cundinamarca (CAR in Spanish)[25], Bogota River is located in the center of Colombia, between the departments of Boyacá and Cundinamarca; it originates in the municipality of Villapinzón and flows into the Magdalena River, covering 347 km with an area of influence of 589,143 hectares, crossing 46 municipalities in total and receiving contributions, in the middle basin, from more than 12 million people, most of whom belong to Bogota.

Given its location, it is very important for the country since the surrounding populations develop economic activities such as agriculture, farming, and some industries have their production plants near the hydrosystem.

Study sampling zone

To determine the presence of the EPs, Puente La Virgen Station (located in 4.795736, -74.095784) and Gibraltar Pumping Station (located in 4.647225, -74.184547) were

taken as study points. Its selection is due to characteristics such as the representation of the beginning and end of the middle Bogota River basin (before and after Bogotá City), and general properties (levels, bathymetry records, discharge of municipal wastewater, surveillance, and security) necessary for sampling.

Drugs of abuse metabolites selection

Metabolites are generated during the metabolic cycle; in the case of drugs of abuse, after the drug is consumed, the body's biochemistry generates reactions involving different molecules to fragment the compound that entered the body and thus promote its elimination through urine, feces, or perspiration. [26–28].

The main metabolites of the drugs of abuse to be identified were selected thanks to the latest reports around the world, Colombia and Bogotá city; the OAS [29] reported an increase in the use of drugs of abuse in the Western Hemisphere, including cannabis, cocaine, ecstasy, methamphetamine and controlled drugs such as methadone.

In Colombia, the National Administrative Department of Statistics (referred to as DANE) [30] where the National Survey of Consumption of Psychoactive Substances (ENCSPA, in spanish) methodology was implemented, reported 2.07% cocaine, 0.69% ecstasy, and methamphetamine use among the surveyed population.

And in the case of Bogotá, the Mayor's Office reported, in 2016, a prevalence of drug use of cocaine (4.34%), ecstasy (1.72%), methamphetamine (0.45%), and non-prescription methadone (0.10%)[31]. These substances were also implemented in recent drug testing studies through the Wastewater-based Epidemiology (WBE) [36, 37] and can be observed in

Table 1~~Table 1~~.

Sampling methods

Two different sampling methods were used: Passive sampling and composite sampling. Fifteen sampling campaigns were proposed at the study sites, each of them from Monday to Friday continuously for the passive sampling and two random dates, according to CAR's methodology, for composite sampling. Sampling has been carried out from January 24 to May 13, 2022.

Passive sampling preconditioning

CFIS is a fully submersible device (see Figure 1~~Figure 1~~) that comprises a small peristaltic pump powered by lithium batteries generating a constant water flow (9.0 mL min^{-1}) that will pass through a selective polyamide cell containing a Empore disk membrane (poly(styrenedivinylbenzene) copolymer) and/or a Gerstel Twister® (polydimethylsiloxane).

While the CFIS was sampling, metabolites of drugs of abuse were stored in a selective cell over time using one empore disk and one Gerstel Twister, and, because it collected the substances using a TWA mode, average concentration data has been obtained for each analyte. In order for the sorbent to be able to retain analytes by overcoming the water surface tension, the disk empore was conditioned in the laboratory, the Gerstel twister did not need conditioning.

The empore disk poly(styrenedivinylbenzene) was conditioned before starting the sampling by immersing it in 50 mL of methanol and then sonicating it for 20 min with an initial temperature of 21.5 ºC; finally, the ultrasound temperature was monitored, and the sorbent was extracted, finding a final temperature of 29.2 ºC and the sorbent in methanol at 34.45 ºC approximately.

CFIS passive sampler. In general, the CFIS device was set up under the previously described indications, as Figure 2~~Figure 2~~ shows, other variables such as sampling time, pump voltage, and particulate fraction were settled thanks to its programmer software. For the purpose of keeping a constant flow through the system, recommended voltage for the correct operation of the pump has been verified in each sampling. The sorbents were installed inside the stainless-steel cell and the equipment was turned on for sampling. Anchors were checked both in the river and on the shore and weights were attached to the device.

Battery voltage, temperature, and motor voltage data were collected inside the unit on a sd memory stick, as well as the temperature of the stainless-steel cell. At the Study sampling zones, a 2 m probe was connected to the nozzle which suctioned water into the CFIS, the probe had a stainless-steel filter at one end in order to separate as many suspended solids as possible that could cause clogging and overloading of the equipment connections.

In addition, at the top of the CFIS casing, the equipment was tied to a riverbank and at the bottom, a 10 kg dead weight was tied to the bottom of the river.

To conclude the installation day, the equipment was deposited in less than 10 min at the sampling point with the help of the remote-control boat.

A total use time of approximately 4 days was determined, from Monday to Friday, with the procedures had been carried out - in most cases - in the morning.

Composite sampling

24-hour composite sampling taking aliquots every 30 minutes simultaneously at each sampling point was done. The containers to collect the sample were amber glass type and a total volume of composition of 1000 ml was estimated. The dates of the

composite sampling were February 25 and April 21, 2022.

Sample preservation and transportation

As for passive sampling and given the implementation of sorbents, the biotransformation potential of the samples decreases as they were not submerged in the interaction medium (water), so they were immediately stored under refrigeration in glassware. After arrival at the laboratory, the sorbents were stored in a refrigerator at 4ºC for one week, and then the analytes were extracted.

For composite sampling, a modification of Castiglioni et al.[38] method was used. Briefly, a cold chain was used for sample transport and no chemical reagents were added for preservation.

Chemicals and chromatographical standards

Empore disk membrane (poly(styrenedivinylbenzene) copolymer) (Cat. No.AH0-3485) was supplied by LABAQUA and ARICEL and a Gerstel Twister® polydimethylsiloxane membranes (Cat. No. 011444-001-00) were provided by Khymos.

As reference materials methamphetamine (Cat. No. 34021), benzoylecgonine (Cat. No. 34016), 3,4-MDMA (Cat. No. 34071), and EDDP (Cat. No. 34069) dissolved in methanol at a concentration of 1 mg mL^{-1} were used and were supplied by Restek. Ammonium formate (HCO_2NH_4) 97 % purity Alfa Aesar, formic acid (HCOOH) 98 % purity Panreac, methanol (CH_3OH) gas chromatography grade, SupraSolv Merck and type 1 water generated at the DLIA facilities were used for the mobile phases.

Samples extraction and concentration

Succinctly, the sorbents were immersed in 3 mL gas chromatography grade methanol, then placed in an ultrasonic bath for 20 minutes and the extract was separated (applied

the same temperature change that was observed in the activation process). The extracts were filtered to 0.22 µm using a PTFE syringe filter and then concentrated to a volume of less than 0.5 mL, and volumetrically gauged to a final volume of 1 mL with gas chromatography grade methanol. Samples were stored in amber vials and refrigerated at 4°C until analysis by UHPLC-MS.

UHPLC – Q Exactive MS

Analyte separation was obtained in a Dionex Ultimate 3000 UHPLC equipment using a Raptor C18 column, 2.7 a catalog No. 9304A62 operated at 25 °C by injecting 1 µL of sample and following the elution gradient described in Table 2~~Table 2~~. Mobile phase A was composed of type 1 water with 4 mM ammonium formate and 0.1 % formic acid. Mobile phase B was composed of gas chromatography-grade methanol, 4 mM ammonium formate, and 0.1 % formic acid.

During the detection and quantification of the analytes, an Orbitrap Q-Exactive was used, configured to the conditions presented in Table 3~~Table 3~~ and equipped with an electrospray ionization source. For all 4 analytes, the protonated ion was tracked.

Quantification method

The ionization source conditions, see Table 4~~Table 4~~, were optimized using a mixture of the four analytes at a concentration of 1 mg L^{-1}, to maximize the Total Ion Current (TIC) and minimize its variation.

The retention times, see Table 5~~Table 5~~ were determined by injecting 1 µL of each analyte at a concentration of 1 mg L^{-1}. Retention times and the exact atomic mass of the protonated substance were used as criteria for analyte identification.

Seven calibration points were generated by injecting 1 µL of a mixture of the analytes at 35, 40, 50, 150, 250, 350, and 400 pg per injection. The area under the curve

and the mass of the analyte were fitted to a linear mathematical model, which was validated by statistical analysis of the correlation coefficient and % residuals. The first curve calibration point (FCCP) was used as reference for the metabolites quantification analysis and it was verified by injecting 7 fortified blanks with a mixture of the analytes at a concentration equivalent to 35 pg per injection.

Comentado [DR1]: Fue evaluado por la inyección de los 7 estándares y se obtuvo un %CV menor al 10%

Analyte recovery evaluation on Empore and Gerstel Twister sorbents

The sorbents were conditioned according to the manufacturer's recommendation and were taken to a solution of 100 mL of type 1 water containing an equivalent mass of $4e^{-4}$ mg of each analyte and were submitted to 3 cycles of 12 hours of agitation in a reciprocal shaker at 120 rpm followed by 12 hours of rest. The procedure of extraction, preparation, and analysis of the samples was carried out. The recovery efficiency was determined as the percentage ratio between the equivalent mass and the recovered mass.

Estimation method for metabolites of drugs of abuse

Since the CFIS is not dependent on flow rate, it was necessary to obtain analyte sampling rates, which are the product of the mass transfer coefficient and the active surface area of the sampler and are obtained from the supplier and from literature and are described in Table 6~~Table 6~~.

Drug mass estimation equations in wastewater were then used using the characterization of marker levels in the collected samples [39], and it was determined using Equation 1 described by Noro et al.[40].

$$C_W = \frac{M_S}{R_S \times t} \quad (1)$$

Using the above equation, the aim was to find the average concentration (C_W in water, pg mL^{-1}) where M_S represented the amount of metabolite retained on the

absorbent (pg) obtained thanks to UHPLC-MS, R_S in (mL day^{-1}), and t (day) is the total exposure time of the CFIS in the river.

Statistical analysis

To analyze which variable (metabolite) had more importance or relevance in the collected samples, in terms of sampling method, concentration, location, or sampling date, a basic multivariate statistical analysis was proposed that also evidenced the importance of using the CFIS sampler as a sampling methodology.

R version 4.2.0 and interface RStudio version 1.2.5042 were used as statistical software for this case.

PCA analysis and correlation analysis using the Spearman test were performed. With the above, as these kinds of samples did not fit a normal distribution, Kruskal-Wallis was used as the non-parametrical test in order to find significant differences between variables applying library and kruskal.test.

Finally, the Wilcoxon test (pairwise.wilcox.test allowed the identification of significant differences between the sampling reported by the CFIS method and the composite sampling with a 95% confidence interval.

Results and discussion

UHPLC- Q Exactive MS analysis

For the case of composite sampling, no masses of metabolites were found in the aliquots collected in any of the sampling campaigns, the chromatographic analysis showed that they were not within the quantification range of analysis proposed in the calibration curve, which was why they were reported as <LOQ.

In theory, for the passive sampling, the analyzed compounds had a certain affinity for certain types of sorbents, Godlewska et al. [15] mention this focusing on Log K_{ow} values with sorbent compatibility, such as the case of BE, MET, and MDMA with the empore disk, thanks to its Log K_{ow} < 4, however, BE retention could be observed in the Gerstel twister in the first sampling in GBR where the empore retained 21851,97 pg mL^{-1} and the Gerstel twister 4675,68 pg mL^{-1}; the same applied for MET with 67101,56 pg mL^{-1} in the empore and 67783,48 pg mL^{-1} in the twister in PT.

On the other hand, EDDP had a theoretical affinity performance with the Gerstel Twister given its Log K_{ow} > 4, and the reported values agree with this, except in the second sampling at GBR, where the empore managed to retain 15908,48 pg mL^{-1} but there was < FCCP in the Gerstel twister.

C_w was set to be null when the concentration was below the FCCP, or the sampling zone was not carried out.

In general, concentration results versus each compound were presented in a Boxplot in Figure 3~~Figure 3~~, BE had more comparative data than ME, MDMA, and EDDP thanks to the occurrence detection of this drug.

At the end of the chromatographic analysis, and as a general representation of chromatographical results, it was possible to observe (in Figure 4~~Figure 4~~ and Figure 5~~Figure 5~~) well-defined peaks with minuscule noise, there was indeed a chromatographical separation and symmetry in all cases. Thanks to the developed chromatographical method, the retention times were optimized, and the top waited time was more or less nine minutes.

Benzoylecgonine

In the case of BE, it was important to mention that 66.6% of the samples showed concentrations above the FCCP the methodology. Thus, the maximum concentration

value corresponded to the first sampling where 21427,3 pg mL^{-1} was obtained, and the minimum C_w was retained in the seventh sampling, in PT with 751,9 pg mL^{-1}. For GBR, the mean C_w value was 7533,8 pg mL^{-1} and for PT is 2270,0 pg mL^{-1}, confirming that GBR was one of the principal spots for analysis showing, from the beginning of the basin to the end, concentration changes due to a greater contribution of discharges to the river by the population of the city.

The results (see Figure 6) showed a clear gap between the PT point, the entrance to the middle basin, and the GBR point. The concentrations increased as the pollutant descended the river path, possibly due to the residual contribution of the citizens to the river and the accumulation.

Methamphetamine and Ecstasy

It was convenient to relate MET and MDMA results since they belonged to the amphetamine family; their effect is usually stimulating and is usually presented in social events that involve considerable amounts of waste of energy. Chromatographical results indicated that TWA occurrences for these metabolites were 16.6% for MET and 20% for MDMA in this study.

Maximum and minimum concentrations for MET were presented in the first campaign sampling with 67101,56 pg mL^{-1} and 191074,78 pg mL^{-1} in PT and GBR, respectively. However, other MET concentration findings were below the FCCP of the chromatographical method.

For MDMA, its maximum concentration was 225844,86 pg $mL^{-1}$and minimum concentration result was 1337,05 pg mL^{-1}; both findings were found in different campaigns, for example, the maximum MDMA TWA concentration was retained in GBR study zone in the first campaign but the minimum value of MDMA TWA concentration was presented in the third campaign, again in GBR.

As well as BE, MET and MDMA TWA concentrations changed over the length of the river from the beginning to the end of the sampling zone (see Figure 7~~Figure 7~~ and Figure 8~~Figure 8~~).

EDDP

Methadone is usually used for pain treatment as a controlled drug, however, it is a controlled drug and its consumption could be derivate from medical conditions and dependence to this drug, as in the case of morphine, oxycodone, or another family of opioids. The case of methadone metabolite was the focus of discussion in the application of suitable sorbents according to the Log K_{ow} coefficient of the compounds. As mentioned before, the optimum sorbent for its retention was the Gerstel Twister, however, better results were obtained with the Empore Disk in this study.

EDDP occurrence was less than 14%, but the sorbents used in passive sampling retained enough molecules in water to had a maximum concentration of 15908,48 pg mL^{-1} and a minimum TWA concentration of 7489,53 pg mL^{-1}.

Statistical analysis

For the purpose of interpreting qualitative variables, sampling weeks where social events were present were cataloged as yes (1) and no (0). Also, the precipitation was classified into the following five levels thanks to CAR meteorological diary reports[41]: 1 – Low (No rain or drizzle of less than 10 mm), 2 – Low-Medium (Rainfall between 10 and 29 mm), 3- Medium (Rainfall between 30 and 59 mm), 4 – Medium-High (Rainfall between 60 and 89 mm) and 5 – High (Rainfall equal to or greater than 90 mm). Sampling dates where only one zone was analyzed and sampling results where <LOQ was obtained, were settled to be zero in order to facilitate the statistical analysis.

A histogram was used to determine, visually, if the results present normality, for that reason, the Shapiro test for normality was performed confirming there was no normality in the BE, MET, MDMA, and EDDP results.

As no homogeneity results were relevant, Kruskal-Wallis test indicated significant differences between BE average concentration (C_w) and Zone with a p-value < 0,05.

The Wilcoxon test allowed us to identify significant differences between the zone and BE average concentration, however, it was not the case for other variables and EPs concentrations.

Correlation tests and PCA were performed. The correlation test (see Figure 10~~Figure 10~~) showed that the EPs concentrations in the river were slightly positively correlated with social events, however, more investigation is needed in order to support this hypothesis; precipitation had a non-proportional linear relationship with BE or MDMA concentrations and the zone correlation relationships showed us there was a non-proportional linear relationship with EPs concentration (particularly for C_w BE) so therefore there was not a linear dependence between PT and GBR and EPs.

On the other hand, PCA percentage explained variances showed us that the first dimension can explain almost 45% of the variances. Therefore, PCA principal contributors were the EPs concentrations and event was the last contributor.

Conclusion

CFIS equipment allowed monitoring these 4 metabolites of drugs of abuse by showing relevant qualities in comparison to the composite sampling because the sorbents accumulated, in several days, higher amounts of metabolites than reported in the composite sampling proposed by CAR methodology.

The method used in passive sampling allowed to reduce field personnel, interferences, and materials in sample collection, and most sampling campaigns, compared to the composite sampling method, were above the FCCP.

Benzoylecgonine TWA concentrations indicated changes from the beginning to the end of the middle Bogotá River basin throughout the different sampling days; likewise, it was the metabolite with the highest detection occurrence compared to the others, which was to be expected since it is one of the most consumed drugs in the city.

Empore disk sorbent performed better than Gerstel Twister because it had a larger contact area with the sampled water. However, the use of Gerstel Twister is not discarded for compounds whose Log K_{ow} value is higher than EDDP using this kind of sampling method.

The metabolites were analyzed in a chromatographic monitoring mode called positive ion, which is why metabolites of drugs of abuse with negative charges in their structure were not included in this study; however, and with what has been observed in these emerging contaminants by using passive sampling with different sorbents, it is possible to adapt the chromatographic method to monitor other substances such as marijuana.

The sampled metabolites under passive sampling showed an increase in concentration as they reached the endpoint of the middle Bogota River basin, this allegedly was assumed since the river is the main catchment point for discharges from the capital city. Since it was possible to observe differences and changes in concentration along the middle basin, it is also possible to track these pollutants even before the discharge of the Bogota River into the Magdalena River.

The correlation test allowed us to observe how meteorological variables such as precipitation could generate diffusion in the pollutant plume and therefore, it is

necessary to consider a low concentration first calibration curve point in a research scope in case of a diffusion situation for some of the metabolites and, subsequently a lowest limit of detection and quantification for the chromatographical method.

The reported results can be implemented in other studies, such as drug consumption through the monitoring of their metabolites by implementing Wastewater-based Epidemiology

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Figure Captions

Tables

Figures

Tables

Table 1. Metabolites of selected drugs of abuse.

Drug of Abuse	IUPAC metabolite name	Metabolite	# CAS
Cocaine	(1S,3S,4R,5R)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-4-carboxylic acid	Benzoylecgonine (BE)	519-09-5
Methamphetamine	N-methyl-1-phenylpropan-2-amine	Methamphetamine (MET)	60124-88-1
Ecstasy	N-methyl-1-(3,4-methylenedioxyphenyl)propan-2-amine	3,4-methylenedioxymethamphetamine (MDMA)	64057-70-1
Methadone	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine	2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	31161-17-8

CAS: Unique numerical identifier assigned by the Chemical Abstracts Service for a specific substance

Table 2. Gradient elution used in the chromatographical method.

Time (min)	Flow (mL min^{-1})	% Mobile phase A	% Mobile phase B
0	0.300	100	0
1.0	0.300	90	10

11.0	0.300	20	80
12.00	0.300	20	80
14.00	0.300	100	0

Table 3. Orbitrap Q-Exactive operating conditions.

Condition	**User Configuration**
Scan Type	FullMS
Scan Range	100.0 - 500.0 m/z
Resolution	17500
Polarity	Positive
AGC Target	1,00E+06

Table 4. Ionization source operating conditions.

Ionization source specification	**User configuration**
Sheath gas flow rate	30
Aux gas flow rate	40
Sweep gas flow rate	0
Spray voltage (kV)	2.50
Capillary temperature (ºC)	300
S-lens RF level (%)	50.0

Table 5. Analytes retention times in the chromatographical developed method.

Metabolite	Rt (min)	Atomic mass (uma)
BE	6.94	290.13868
MET	5.54	150.12773
MDMA	5.75	194.11756
EDDP	9.30	279.19815

Rt: Retention time (min).

Table 6. Metabolite analytical information

Metabolite	Boiling point at 1 atm (ºC)	Log K_{ow}	Rs (mL day^{-1})	Excreted (%)
BE	442.4±45.0	-1.32	12.81	45%
MET	215.5±9.0	2.22	1.12*	43%
MDMA	283.4±9.0	2.28	1.12*	65%
EDDP	393.9±42.0	4.94	5,92*	30.9%

Boiling Point: Obtained by Chem Spider platform.

Log K_{ow}: Octanol-Water partition coefficient, obtained by Chem Spider platform.

Rs: Sampling Rate [mL day^{-1}] *Approximate value

Excreted %: the percentage of the analyte after its metabolic degradation that is eliminated through urine, perspiration, feces, etc. Values cited from Garcia-Lor et al. [42] y Foppe et al. [43].

Figures

SUPPORTING MATERIALS

Figure 1. Principal parts of the CFIS. Filter, 2. Absorbents, 3. peristaltic pump, 4. electronic card, 5. batteries, and 6. filter. Adapted from Labaqua [44]

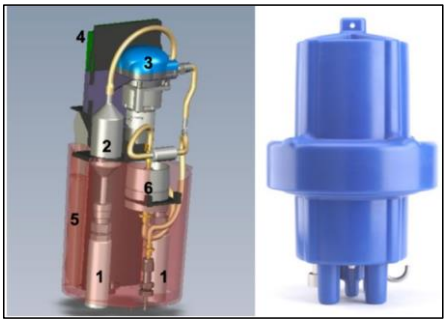


Fig. 1

Figure 2. CFIS in the laboratory before the sampling campaign.

Fig. 2

Figure 3. Boxplot diagram identifying general differences between TWA concentrations and metabolites.

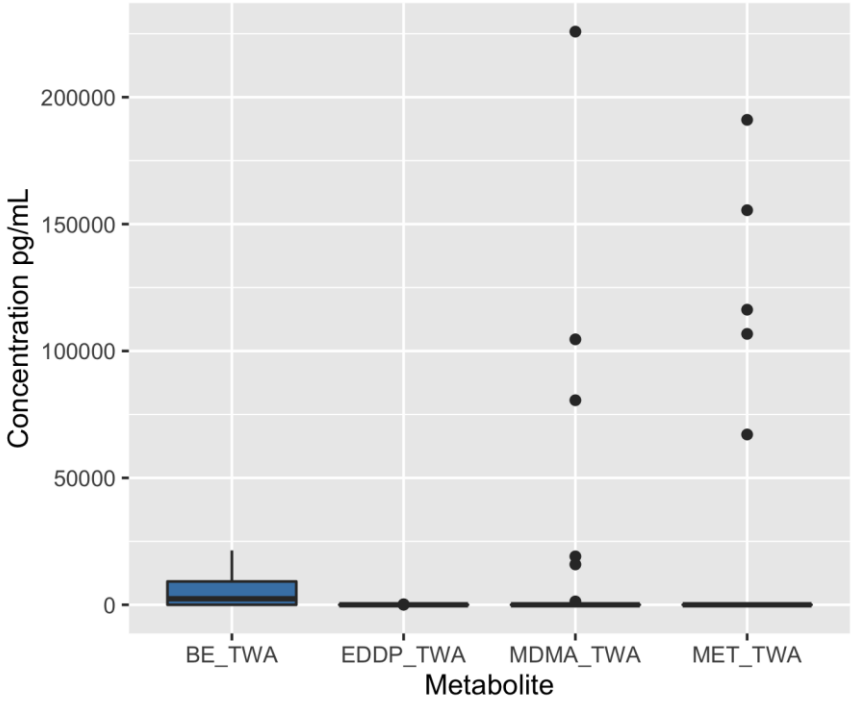
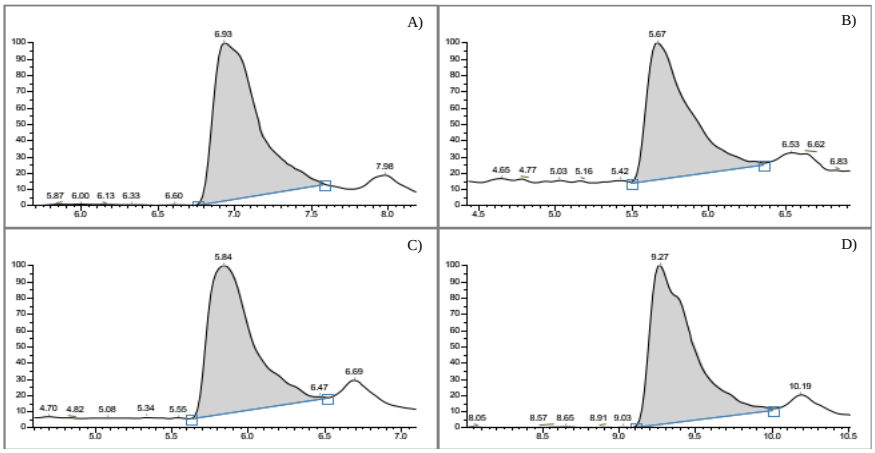


Fig. 3

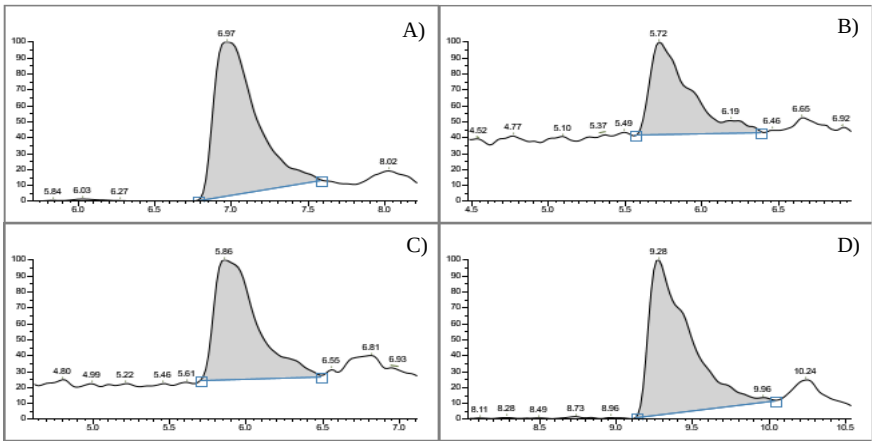
Figure 4. Chromatograms for the first sampling campaign analysing Empore Disk sorbent in GBR.



A) BE, B) MET, C) MDMA, D) EDDP. X-axes represent Rt, Y axes represent relative abundance percentage (%)

Fig. 4

Figure 5. Chromatograms for the first sampling campaign analysing Gerstel Twister® sorbent in GBR.



A) BE, B) MET, C) MDMA, D) EDDP. X-axes represent Rt, Y axes represent relative abundance percentage (%)

Fig. 5

Figure 6. Benzoylecgonine concentrations over time.

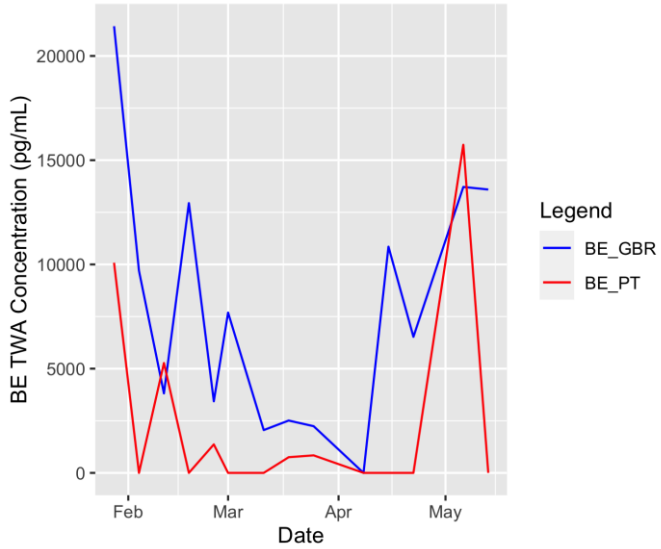


Fig. 6

Figure 7. Methamphetamine concentrations over time.

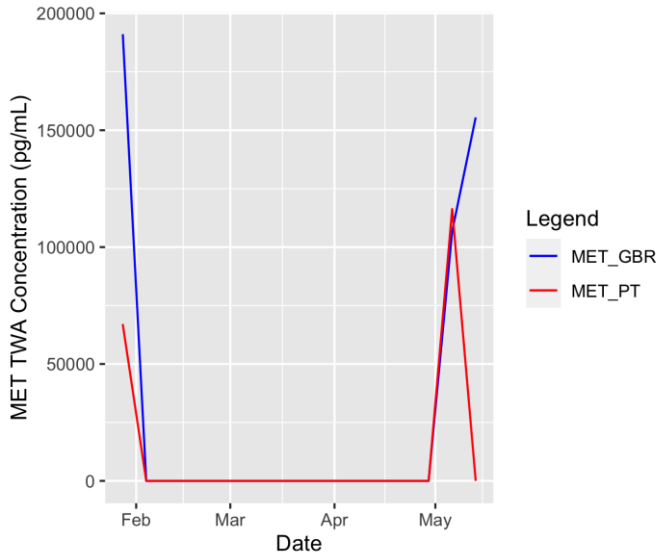


Fig. 7

Figure 8. Ecstasy concentrations over time.

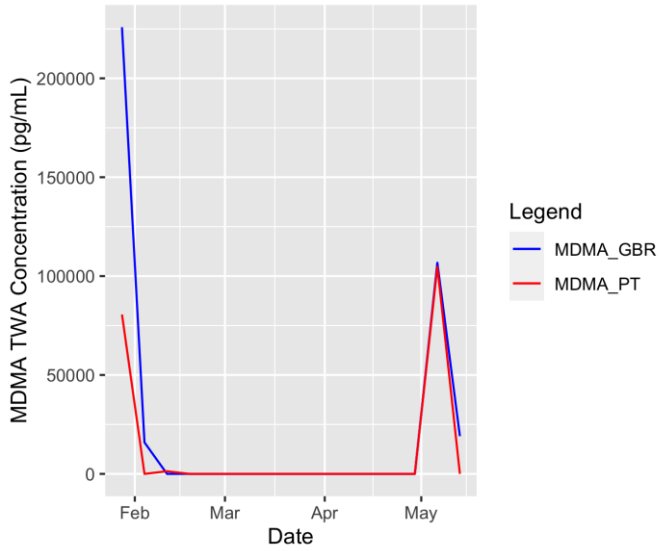


Fig. 8

Figure 9. EDDP concentrations over time.

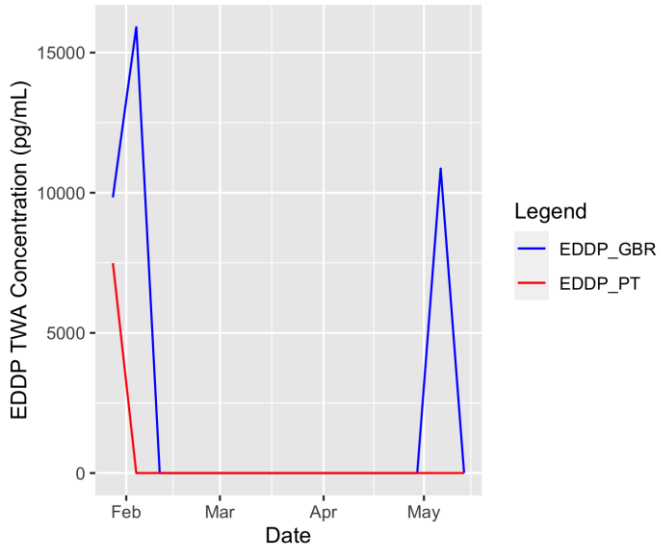
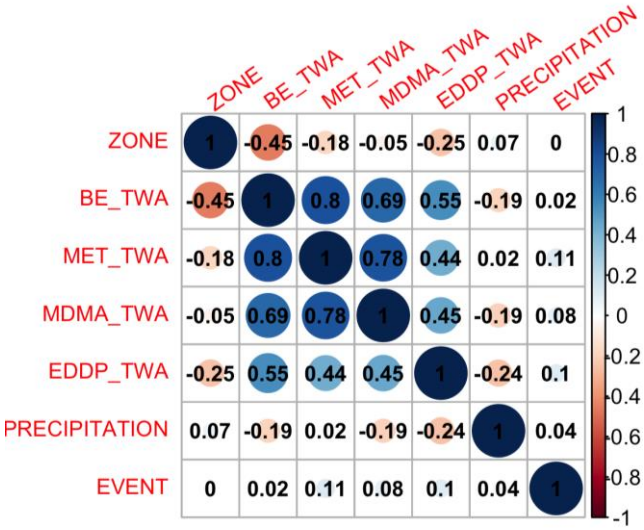


Fig. 9

Figure 10. Correlation matrix for EPs concentrations using the CFIS device versus external variables.



Negative values represent a non-proportional relationship between variables.

Fig. 10